Humanity is cutting down its forests apparently oblivious to the fact that we may not be able to live without them.

Isaac Asimov

Isaac Asimov's Book of Science and Nature Quotations, 1988

Forum

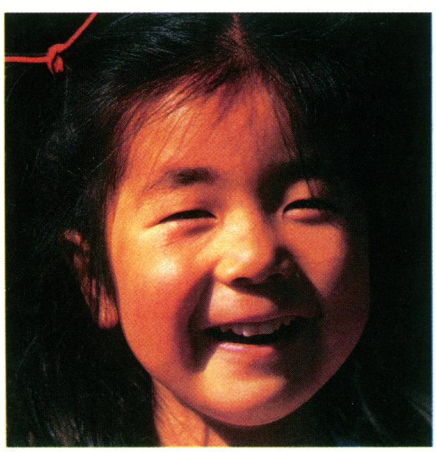
Fluoridation Debate

Citizens for Safe Drinking Water, a San Diego, California, group opposed to fluoridation as a means of preventing tooth decay, is trying to repeal the state's 1995 mandatory fluoridation law by gathering enough signatures to place an initiative before the voters. David Kennedy, a biochemist and part-time dentist in San Diego who is also president of the 4,000-member organization, points to studies concluding that fluoridation causes health problems and doesn't reduce tooth decay.

Among Kennedy's supporters is EPA chemist William Hirzy, who is backing the initiative in his capacity as an officer in the National Federation of Federal Employees. Arrayed against Kennedy and others opposed to fluoridation are scientists who claim fluoridated water is beneficial and nontoxic.

The practice of adding fluoride to tap water began in 1945. In the United States today, the tap water for approximately 134 million people has fluoride added to it, according to the CDC, and about 10 million people drink water that naturally contains the chemical. The reported optimal amount of fluoride is between 0.7 and 1.2 parts per million. A 1991 Public Health Service report credited community water fluoridation as being responsible for 20–40% fewer dental cavities compared with nonfluoridated areas.

Though fluoride can be directly applied to teeth or used in mouth rinses, community water fluoridation is "a remarkably efficient way of controlling dental caries at the community level," says Lawrence Furman, a dentist and scientist at the National Institute of Dental Research. "Fluoridated water reaches everybody, that's why we need it," argues epidemiologist Brian Burt of the University of Michigan in Ann Arbor. But making fluoride so ubiquitous bothers opponents. Kennedy cites a number of studies that conclude that drinking fluoridated water is responsible for increased hip fractures. Some research indicates that fluoride may build up in bones over a lifetime and make them brittle, leaving the elderly particularly at risk for fractures. Other studies, however, have not supported this conclusion.



Unsafe smiles? A California-based group is pushing to repeal mandatory fluoridation laws, citing health concerns.

But studies on both sides of the issue have flaws, says Thomas Reeves, a fluoridation engineer at the CDC. For instance, a 10-year study reported in the 12 August 1992 issue of the *Journal of the American Medical Association* compared the incidence of hip fractures in residents of a fluoridated locality with the incidence in two nonfluoridated localities. The researchers concluded there was a link between water fluoridation and the fractures. The conclusion, Reeves says, is flawed for 3 of the 10 years because fluoride was not actually added to water in the so-called fluoridated locality. Furthermore, the studies show only slight differences in either direction, he said, adding that the CDC's official position is that more research is needed. But some say that the most compelling weakness of the *JAMA* study is the fact that it is an ecologic study, in which the characteristics of the group as a whole, rather than those of the individual constituents of the group, were studied, conceivably leading to the derivation of inaccurate inferences.

Fluoridation opponents point to an even more dire alleged consequence of fluoridating water—osteosarcoma, a form of bone cancer. They cite studies done in New Jersey as linking fluoridated water with this rare cancer in the United States. But Burt argues those analyses are statistically faulty. And the most recent animal studies performed by the National Toxicology Program failed to find a link.

Another point of contention raised by fluoridation opponents is dental fluorosis, the mottling of teeth that can result from drinking water containing fluoride from multiple sources. While Kennedy argues that dental fluorosis is a marker for serious internal problems caused by fluoride, Reeves, Furman, and other fluoride proponents disagree and state that the mild dental fluorosis that can occur from drinking fluoridated water is often detectable only by trained dentists. Reeves does acknowledge that, because fluoridated water is used in many products that people eat and drink, the incidence of dental fluorosis has been increasing. "We're seeing [dental fluorosis in] 7, 8, and 10% [of the population]," says Reeves. "A certain percent of the population may be getting too much."

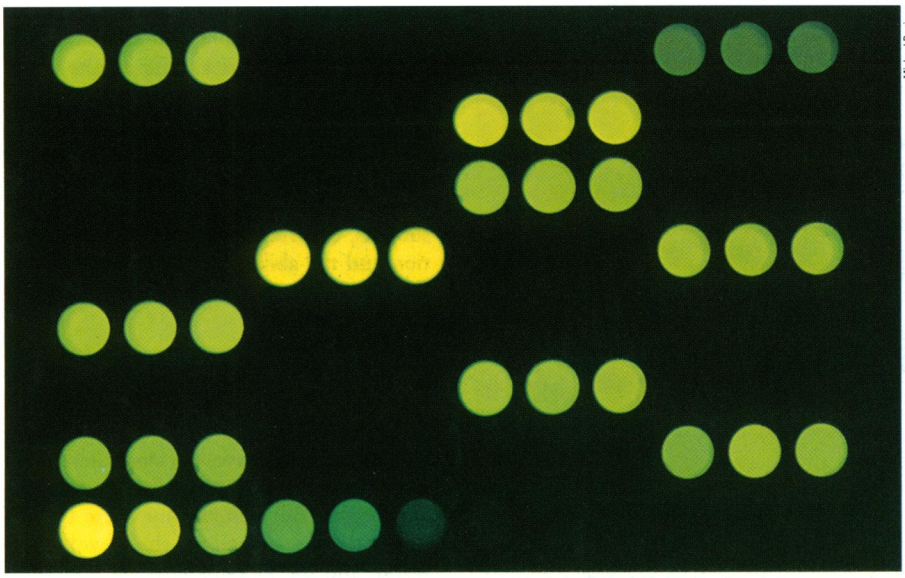
Some scientists, says Reeves, suspect the high fluoride content of many toothpastes is a major reason for dental fluorosis. There is some discussion among fluoride specialists, he says, of lowering the dose in toothpastes aimed at young children for this reason, and also because some scientists worry that young children who swallow fluoride toothpaste may become ill.

The opposite concern—that children are getting too little fluoride—has also surfaced. A number of dental specialists fear the increased consumption of nonfluoridated bottled water may lead to increases in dental cavities. But there are currently no data to support this contention. Meanwhile, the debate over fluoridation of water continues.

An Enlightened Approach to Screening for Dioxins

Scientists recently had a bright idea about how to screen for environmental toxins. Researchers at the University of California at Davis have developed a bioassay system to detect polyhalogenated aromatic hydrocarbons such as dioxins in environmental samples. Dubbed the CALUX (for chemically activated luciferase gene expression) system, the assay is based on recombinant cell lines into which researchers have inserted the firefly luciferase gene. When exposed to dioxin-like compounds, the recombinant cells luminesce.

Polyhalogenated aromatic hydrocarbons are a diverse group of compounds that are widespread in the environment. Exposure to these compounds can lead to carcinogenesis, liver toxicity, birth defects, damage to the immune system, skin lesions, and even death. "Given the ubiquitous presence of these toxic



Michael Denison

The glow gives it away. A new assay uses the firefly luciferase reporter gene, which luminesces in the presence of the Ah receptor, to test for the presence of dioxins in environmental samples.

compounds in the environment, there is a need for a rapid, inexpensive screening assay to monitor toxic output at a given site, to detect the presence of these chemicals in individuals who work in such environments, and to test sites in which these chemicals are believed to be deposited," says Michael Denison, professor of environmental toxicology at the University of California at Davis and one of the assay's inventors.

Denison and his colleagues have studied human, rat, guinea pig, hamster, and mouse cells, and are currently experimenting with a fish cell line. Testing is carried out by placing the environmental specimen in a test plate with the recombinant cells. The cells contain the luciferase reporter gene, which is linked to a DNA sequence called a dioxin-responsive element (DRE). The DRE is the binding site for the dioxin-activated aryl hydrocarbon receptor (AhR), a cell protein that mediates the toxic effects of dioxins. When the cells are exposed to dioxin-like compounds present in environmental samples, the AhR is activated and stimulates expression of the luciferase gene via the DRE. Luciferase can be easily measured because it emits light.

EPA regulations require an assay of the concentration of individual dioxin-like compounds in an environmental sample using high-resolution gas chromatography coupled with high-resolution mass spectrometry. Results for the individual compounds are then multiplied by a toxic equivalency factor to arrive at the total toxic equivalency for the mixture of toxins. This is a slow, cumbersome, and expensive process. The CALUX system detects the presence of such chemicals, but does not indicate which individual chemical or combination is present. Still, it is a rapid and inexpensive screening method, Denison explains.

"This [system] is a natural progression from the current bioassay," says Denison. "The previous bioassay, [using] the H4IIE wild-type cells, lacks much of the selectivity and sensitivity that the CALUX system has. An advantage or our system is that luciferase reporter activity is unaffected by chemicals that are known to inhibit activity in the H4IIE assay."

The bioassay is so promising that it is now the object of a commercial enterprise. George Clark, president of Xenobiotic Detection Systems, Inc. in Durham, North Carolina, is marketing the assay to environmental researchers. "Currently we're offering analyses of blood, serum, milk, water, or sediment in parts per trillion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents. We're seeking regulatory approval for our assay as a screening method that can be confirmed by gas chromatography–mass spectrometry, which should provide significant savings in the analysis of the toxicity of this class of compounds," Clark says.

The bioassay is not a test that can currently be run in the field, but specimens can be collected, frozen, and forwarded to the laboratory for assay. "However," says Clark, "we're developing a mobile laboratory that can be parked at the site of investigation in areas that require a large number of tests."

"The strong point of this assay is its speed and potential as a screening method," according to Scott Masten, a fellow in the Environmental Toxicology Program at the NIEHS who is familiar with the assay. "Using the chromatograph–spectrometer method you could assay perhaps two dozen specimens in a week. This bioassay can screen a hundred specimens in a week and only those that show activity, that luminesce, need to be run through the chromatograph and spectrometer. The bioassay will be even faster if it is automated, which can be done. The major disadvantage of the CALUX method is the problem with specificity. It measures total dioxin-like activity in the specimen without indicating which chemicals are present. The active sample still must be analyzed by chromatograph and spectrometer, but even so, the bioassay is around 20% the cost of the chromatograph–spectrometer."

A New Way to Control Experiments

In case–control studies in which a researcher is trying to determine how genetics and environmental exposure interact to cause illness, the job of being a control is not very appealing. Controls must fill out questionnaires that will determine if they have been exposed to the environmental agent, supply tissue samples for the genetic analysis (which normally involves a needle prick), and perhaps suffer the anxiety of questions raised by the genetic analysis itself, including how they will be affected if the analysis shows a genetic susceptibility to illness. For study subjects that have a disease, there is at least the hope that the research will lead to a new treatment for their ailment, but for the healthy control even this comfort is missing. It is not unusual for many controls to quit in the middle of a study on genetic susceptibility to environmental exposures. Sometimes so many quit that the study loses its validity.

However, according to two NIEHS research statisticians, in most studies of this type, the effect of the gene–exposure interaction can be found without using a control group. David Umbach and Clarice Weinberg report in the 15 August 1997 issue of *Statistics in Medicine* that if two assumptions can be made—that the disease or condition under investigation is rare in the general population and that the exposure of interest and the genetic condition are not statistically related—mathematical analysis of the gene–exposure interaction can progress with no data from controls at all.

The drawback to this method is that without data on the exposure history or on the genetic makeup of a control group, only the combined effect of the susceptible genotype and the exposure can be found. Neither the effect of the exposure alone nor of the genotype alone can be assessed.

Since it is less difficult (and less expensive) to convince a group of controls to fill